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THE CYTOKINE FactsBook

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SECOND EDITION

THE CYTOKINE *FactsBook*

Second Edition

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Contents

IL-1

Other names
Lymphocyte-activating factor (LAF), endogenous pyrogen (EP), leukocyte endogenous mediator (LEM), mononuclear cell factor (MCF), catabolin.

THE MOLECULES

Interleukin 1 (IL-1) has a very wide range of biological activities on many different target cell types including B cells, T cells and monocytes[1–3]. *In vivo*, it induces hypotension, fever, weight loss, neutrophilia and acute phase response. IL-1α and IL-1β are distinct molecular forms of IL-1 derived from two different genes. IL-1α is mostly cell associated and IL-1β is mostly secreted. Although the amino acid sequence homology between the α and β forms is only about 20%, these molecules bind to the same receptor and have very similar if not identical biological properties. An IL-1 receptor antagonist (IL-1Ra) has been described which is structurally related to IL-1β and binds to the IL-1 receptor[4]. Intracellular forms of human IL-1Ra have also been identified that are splice variants of IL-1Ra[5]. The antagonist is made by the same cells that secrete IL-1 and may be an important physiological regulator. A cysteine protease (converting enzyme) which releases mature IL-1β has also been cloned and is termed caspase-1, since it is the founder member of the caspase family of cysteine proteases[6,7]. A cowpox virus-derived inhibitor (CRMA) of the IL-1-converting enzyme has been shown to inhibit the host inflammatory response[8].

Crossreactivity
There is 62% amino acid sequence homology between human and mouse IL-1α and 68% for IL-1β. Both forms crossreact between humans and mice. There is 77% sequence homology between human and mouse IL-1Ra.

Sources
A wide variety of cells secrete IL-1, including monocytes, tissue macrophages, Langerhans cells, dendritic cells, T lymphocytes, B lymphocytes, natural killer (NK) cells, large granular lymphocytes (LGL), vascular endothelium and smooth muscle, fibroblasts, thymic epithelia, astrocytes, microglia, glioma cells, keratinocytes and chondrocytes.

Bioassays
Activation of murine thymocytes or murine T cell lines. PGE_2 induction in fibroblasts using an IL-1-neutralizing antibody as control. *In vivo* (rabbit) pyrogen assay.

Physicochemical properties of IL-1α and IL-1β

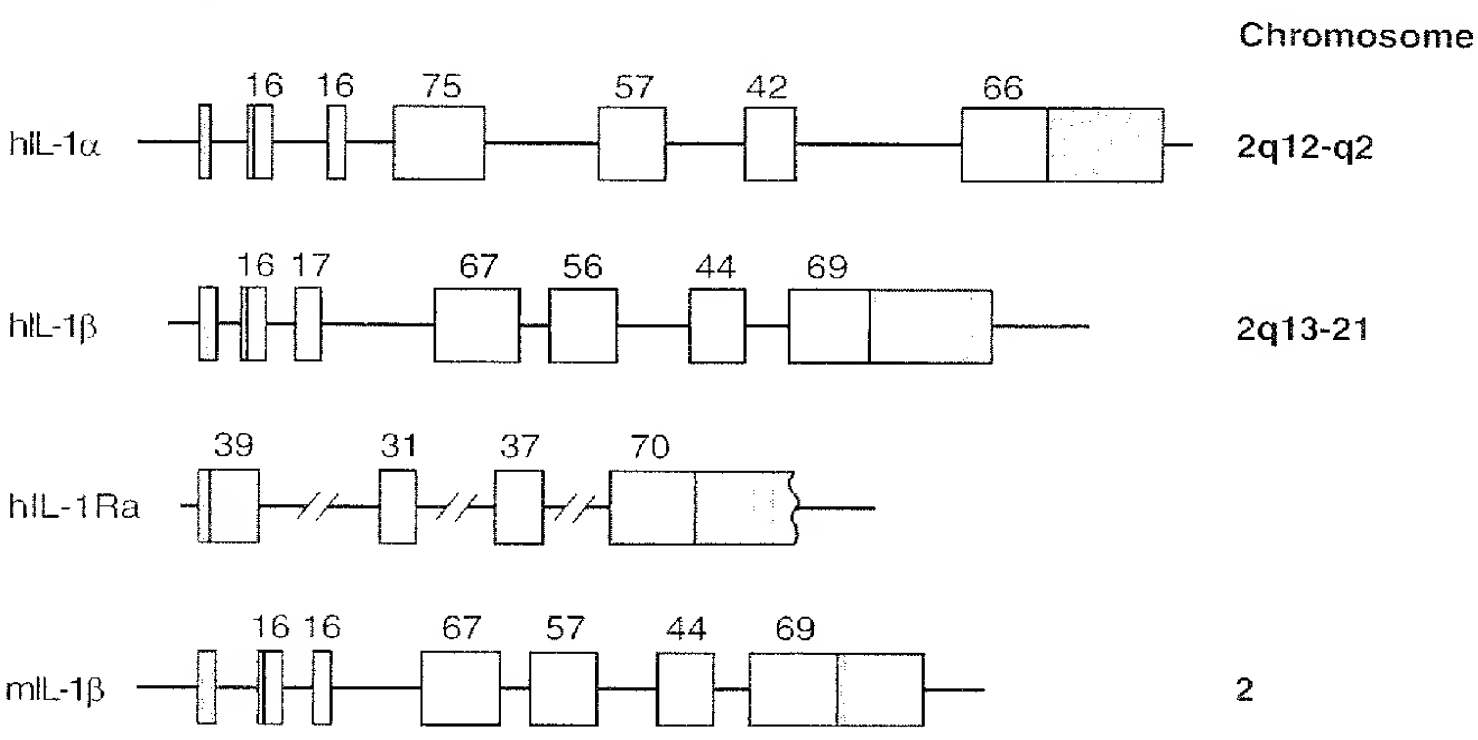
Property	IL-1α		IL-1β	
	Human	Mouse	Human	Mouse
pI	5	5	7	7
Amino acids – precursor	271	270	269	269
– mature[a]	159	156	153	159
M_r (K) – predicted	18.0	18.0	17.4	17.4
– expressed	17.5	17.4	17.3	17.5
Potential *N*-linked glycosylation sites[b]	2	3	1	2
Disulfide bonds	0	0	0	0

[a] After proteolytic removal of propeptide.
[b] IL-1 is not normally glycosylated.

3-D structure

The structure of IL-1α has been determined at a resolution of 2.7 Å by X-ray crystallography and IL-1β at lower resolution by NMR spectroscopy. Both forms of IL-1 are stable tetrahedral globular proteins formed by an antiparallel six-stranded barrel closed at one end by a six-stranded β-sheet to form a bowl-like structure.

Gene structure[11–14]



Other names
Mast cell growth factor (MCGF), multi-colony stimulating factor (multi-CSF), eosinophil-CSF (E-CSF), haematopoietic cell growth factor (HCGF), burst-promoting activity (BPA), P-cell stimulating factor activity, thy-1 inducing factor, WEHI-3 growth factor.

THE MOLECULE

Interleukin 3 (IL-3) is a haematopoietic growth factor which stimulates colony formation of erythroid, megakaryocyte, neutrophil, eosinophil, basophil, mast cell and monocytic lineages[1]. IL-3 may also stimulate multipotent progenitor cells, but it is more likely to be important in committing progenitor cells to a differentiation pathway rather than self-renewal of primitive stem cells. Many of the activities of IL-3 are enhanced or depend upon costimulation with other cytokines. IL-3 does not stimulate lymphocyte colony formation, but it is a growth factor for B lymphocytes and it activates monocytes, suggesting that it may have an additional immunoregulatory role. IL-3 has been used clinically to expand haematopoietic precursors after bone marrow transplantation, aplastic anaemia and chemotherapy[2].

Crossreactivity
Amino acid sequence homology between mouse and human IL-3 is only 29% and there is no cross-species reactivity.

Sources
Activated T cells, mast cells, eosinophils.

Bioassays
Proliferation of TF-1 (human erythroleukaemia), MO7e (human megakaryoblastic leukaemia) or AML-193 (acute myeloid leukaemia) cell lines. Stimulation of erythroid, granulocyte and macrophage colony formation in bone marrow colony assay.

Physicochemical properties of IL-3

Property	Human	Mouse
pI	4–8	4–8
Amino acids – precursor	152	166
– mature[a]	133	140
M_r (K) – predicted	15.1	15.7
– expressed	14–30	28
Potential *N*-linked glycosylation sites	2	4[b]
Disulfide bonds	1	2

[a] After removal of predicted signal peptide.
[b] Glycosylation only at positions 16 and 86 (see sequence). Glycosylation is not required for biological activity.

IL-4

Other names
B cell-stimulating factor 1 (BSF-1).

THE MOLECULE

Interleukin 4 (IL-4) is a pleiotropic cytokine derived from T cells and mast cells with multiple biological effects on B cells, T cells and many nonlymphoid cells including monocytes, endothelial cells and fibroblasts. It also induces secretion of IgG1 and IgE by mouse B cells and IgG4 and IgE by human B cells. The IL-4-dependent production of IgE and possibly IgG1 and IgG4 is due to IL-4-induced isotype switching[1–3]. IL-4 appears to share this property with IL-13.

Crossreactivity
Two regions of human IL-4 (amino acids 1–90 and 129–149) share approximately 50% sequence homology with the corresponding regions of mouse IL-4. In contrast, the region from amino acid positions 91–128 shares very little homology with the corresponding region of mouse IL-4. There is no cross-species reactivity between human and mouse IL-4.

Sources
Mast cells, T cells, some mouse B-cell lymphomas, bone marrow stromal cells.

Bioassays
Human: Proliferation of PHA T-cell blasts in the presence of blocking anti-IL-2 or anti-IL-2R antibody; proliferation of MO7 cell line; increased expression of CD23 or surface IgM on human tonsillar B cells.

Mouse: Proliferation of CTLL in the presence of anti-IL-2 or anti-IL-2R antibody. Increased expression of MHC class II on murine B cells.

Physicochemical properties of IL-4

Property	Human	Mouse
pI	10.5	6.5
Amino acids – precursor	153	140
– mature[a]	129	120
M_r (K) – predicted	15.0	13.6
– expressed	15–19	15–19
Potential *N*-linked glycosylation sites	2[b]	3
Disulfide bonds	3	3

[a] After removal of signal peptide.
[b] Asn38 is glycosylated.

IL-5

Other names
Eosinophil differentiation factor (EDF), eosinophil colony-stimulating factor (E-CSF), B cell growth factor II (BCGFII), B cell differentiation factor for IgM (BCDFμ), IgA enhancing factor, T cell-replacing factor (TRF).

THE MOLECULE

Interleukin 5 (IL-5) is a T cell-derived glycoprotein which stimulates eosinophil colony formation and is an eosinophil differentiation factor in humans and mice. It is also a growth and differentiation factor for mouse but not human B cells[1–3].

Crossreactivity
There is 71% homology between mouse and human IL-5 and significant cross-reactivity in functional assays.

Sources
Mast cells, T cells and eosinophils.

Bioassays
Human: Eosinophil differentiation; proliferation of TF1 cell line.

Mouse: Eosinophil differentiation; proliferation of BCL1 or B13 B-cell lines.

Physicochemical properties of IL-5

Property	Human	Mouse
pI (calculated)	7	7.8
Amino acids – precursor	134	133
– mature[a]	115	113
M_r (K) – predicted	13.1	13.1
– expressed[b]	45	40–50
Potential *N*-linked glycosylation sites	2	3
Disulfide bonds[c]	2	2

[a] After removal of predicted signal peptide.
[b] Homodimer.
[c] Interchain.

3-D structure
IL-5 is an antiparallel disulfide-linked homodimer. The monomer is biologically inactive. The structure of the dimer has been determined at a resolution of 2.4 Å[4]. It has a novel two-domain structure with each domain showing significant structural homology to the cytokine fold in GM-CSF, M-CSF, IL-2, IL-4 and growth hormone. The IL-5 structure is made up of two left-handed bundles of four α-helices with two short β-sheets on opposite sides of the molecule. The C-terminal strand and helix of one chain of the dimer together with three helices and one strand at the N-terminal end of the other chain make up the bundle of four helices and a β-sheet. This dimeric structure of IL-5 is unique. A 3-D image and PDB file are available from SwissProt P05113.

Other names

Interferon-β2 (IFNβ2), 26-kDa protein, B cell-stimulatory factor 2 (BSF-2), hybridoma/plasmacytoma growth factor (HPGF or IL-HP1), hepatocyte-stimulating factor (HSF), monocyte granulocyte inducer type 2 (MGI-2), cytotoxic T cell-differentiation factor and thrombopoietin.

THE MOLECULE

Interleukin 6 (IL-6) is a multifunctional cytokine secreted by both lymphoid and nonlymphoid cells which regulates B and T cell function, haematopoiesis and acute phase reactions[1–3].

Crossreactivity

There is 42% homology between mouse and human IL-6. Human IL-6 is functional on mouse cells but mouse IL-6 has no activity on human cells.

Sources

IL-6 is made by lymphoid cells (T cells, B cells), and many nonlymphoid cells, including macrophages, bone marrow stromal cells, fibroblasts, keratinocytes, mesangium cells, astrocytes and endothelial cells.

Bioassays

Proliferation by IL-6-dependent B9 cell line. Increased Ig secretion by CESS or other EBV-transformed human lymphoblastoid B cell lines.

Physicochemical properties of IL-6

Property	Human	Mouse
pI (calculated)	6.2	6.5
Amino acids – precursor	212	211
– mature[a]	183[b]	187
M_r (K) – predicted	20.8	21.7
– expressed	26	22-29
Potential *N*-linked glycosylation sites	2	0[c]
Disulfide bonds[d]	2	2[d]

[a] After removal of predicted signal peptide.
[b] N-terminal amino acids of human IL-6 derived from a T-cell line, an osteosarcoma cell line and a liposarcoma cell line are Pro, Ala and Val respectively, indicating heterogeneity in the signal peptide cleavage site.

3-D structure

IL-6 has a four antiparallel α-helical structure similar to IL-11, LIF, OSM and GM-CSF. A 3-D image and PDB file are available from SwissProt P05231.